REMARKS

Rejections Under 35 USC § 103(a)

In the Office Action, dated August 23, 2006, the Examiner rejected Claims 1, 3-5, 9-17, 19-20, 22, 24-40, 51, 53-57, 59-64, 69-74, and 76-91 under 35 USC § 103(a) as obvious over the primary reference EP 0 431 905 A1 ("Ogawa") and further in view of Pfister et al., *J. Biol. Chem., 271(3)*: 1687-1694 (1996) and U.S. Patent No. 5,234,809 ("the Boom patent") for reasons previously given in this record. The Pfister reference is cited by the Examiner as an example of the RNeasy® products and protocols (Qiagen GmbH, Hilden, Germany). Applicants respectfully traverse the rejections for the reasons explained below.

To clearly explain the difference between Applicants' claimed invention and the references cited by the Examiner, this response includes a Declaration Pursuant to 37 CFR § 1.132 by co-inventor Uwe Oelmüller, attached as Exhibit 1. Dr. Oelmüller has extensive experience in the use and development of methods of separating and isolating biomolecules and is well familiar with the method described in the Ogawa, the RNeasy® products and protocols as mentioned in the Pfister reference, and the methods described in the Boom patent.

Ogawa describes the purification of phage DNA from denatured and decomposed proteins and smaller molecules using an ultrafiltration membrane. As Dr. Oelmüller explains in paragraphs 5 and 6 of his declaration, ultrafiltration is a biochemical technique that separates molecules on the basis of size (size discrimination, size exclusion). An ultrafiltration membrane (also referred to as an "ultrafilter") separates biomolecules on the basis of whether or not a particular biomolecule is sufficiently small to pass through the pores of a particular ultrafiltration membrane or sufficiently large not to pass through the pores of the ultrafilter. Thus, ultrafiltration can only purify nucleic acid molecules from other types of molecules (e.g., proteins, carbohydrates, lipids) in a complex mixture if the individual nucleic acid molecules are larger than the size of the pores of a particular ultrafiltration membrane *and* the other types of molecules in the mixtures are sufficiently small to pass through the pores of the same ultrafiltration membrane. This is clearly shown in the example described in Ogawa, where phage DNA molecules released from phage particles are retained on a chosen ultrafiltration membrane surface because the DNA molecules are too large to pass through the pores of that membrane,

whereas smaller molecules such as decomposed and denatured phage proteins are sufficiently small to pass through the pores of the membrane (see, column 4, lines 23-34, of Ogawa). Clearly, selection of the appropriate size-excluding ultrafiltration membrane is critical to success in Ogawa or any other protocol that relies on ultrafiltration. Ogawa specifically teaches that an ultrafiltration membrane useful in the method preferably has a fractionation molecular weight of 20,000 (daltons) to 1,000,000 (daltons) (see, column 3, lines 31-34, of Ogawa). The working example in Ogawa employed an ultrafiltration membrane that had a fractionation molecular weight of 300,000 (daltons) and that effectively retained the relatively large phage DNA molecules but allowed the smaller decomposed and denatured phage proteins and other smaller molecules to pass through as waste (see, column 4, lines 23-39, of Ogawa).

Dr. Oelmüller also explains in his accompanying declaration that the recommendations for selecting an ultrafiltration membrane with a particular molecular weight fractionation in Ogawa are reasonable in view of the known approximate size of phage DNA molecules and the sizes of denatured/decomposed proteins and other molecules likely to be in a sample of M13 phage particles prepared from an infected culture of *E. coli* bacteria and also in view of the guidelines and recommendations that are provided by manufacturers of ultrafiltration membranes, such as the Pall Corporation (see, paragraphs 7-10, of the attached declaration). For example, text and tables from the website of the Pall Corporation (attached at Tab B in the Oelmüller declaration) and a table entitled "Relative Partikelgrößen" (i.e., "Relative Particle Size") from the Pall Gelman Sciences catalog of 1998 (attached at Tab C in the Oelmüller declaration) provide useful information and guidelines for selecting ultrafiltration membranes for separating biomolecules, such as nucleic acids and proteins. The table from the Pall Gelman Sciences catalog of 1998 shows that ultrafiltration membranes used in separating biomolecules may have pore sizes in the range of 10 angstroms (0.001 μm) to 1000 angstroms (0.100 μm) to separate species ranging in molecular weights from 10,000 daltons to 1,000,000 daltons. An ultrafiltration membrane with a molecular weight cut-off (MWCO) of 300,000 (daltons), as used in Ogawa, has a pore size of about 0.035 μm (see, Table 3 at Tab B and the table of "Relative Partikelgrößen" in Tab C, of Oelmüller declaration).

Membranes with pore sizes greater that 0.100 μm are quite large with respect to the size of biomolecules. In fact, according to the table of "Relative Partikelgrößen" in Tab C of the

Oelmüller declaration, membranes that have pore sizes greater that 0.100 μm are not considered as "ultrafiltration" membranes that can be used to separate biomolecules. Thus, as noted by Dr. Oelmüller, membranes having pore sizes of 0.22 μm and 0.45 μm are typically employed in the art to filter out intact bacterial cells or cell fragments from a liquid sample. For example, Ogawa used a membrane having a pore size of 0.45 μm to filter out *E. coli* cells from a liquid culture medium containing *E. coli* cells and M13 phage particles (see, paragraph 9, of the Oelmüller declaration; column 4, lines 20-22, of Ogawa). Clearly, persons skilled in this art understand that membranes having a pore size that is greater than 0.100 μm are not useful for separating various sizes of biomolecules by size exclusion because such pore sizes are simply too large to prevent individual biomolecules from passing through such membranes (see, also, paragraph 10, of the Oelmüller declaration). As also explained by Dr. Oelmüller regarding membranes having pores sizes at the other end of the range for ultrafiltration membranes, the utility of such ultrafilters can be quite limited owing to the fact that such pore sizes may be simply too small to be useful for separating a complex mixture of biomolecules by size exclusion. For example, cell lysates may contain impurities such as cell fragments, organelles, and/or various molecular aggregates that are sufficiently large to block or clog such membranes and/or entrap desired target nucleic acid molecules within such impurities that are present on the membrane surface. In addition, many proteins are considerably larger than the 10,000 dalton MWCO of an ultrafiltration membrane having a pore size as small as 0.001 μm (see, paragraph 11, of the Oelmüller declaration).

As Dr. Oelmüller explains in his declaration, he and his co-inventors recognized that a process for isolating and purifying nucleic acids that permits use of highly porous, non-siliceous membranes that have pore sizes that are sufficiently large to allow cell fragments, proteins, and other undesired impurities to wash through while retaining the desired target nucleic acid molecules on the same side of the membrane to which they were initially applied and bound, would be a significant advantage over other methods, including previously known ultrafiltration methods. The process according to this invention clearly provides this advantage over the prior art *without* being restricted to the limited (i.e., 0.100 μm or smaller) pore sizes of ultrafiltration membranes. In fact, the process of Applicants' invention typically provides relatively high yields of nucleic acids from cell lysates and other nucleic acid-containing mixtures using membranes

that have pores sizes that are clearly larger than those employed in ultrafiltration to separate biomolecules by size exclusion. For example, Table 5 at page 25 (Example 5) of the specification, presents surprising data obtained using the process of the invention to isolate RNA from lysates of HeLa cells. As shown in Table 5, excellent yields of RNA were obtained from the HeLa cell lysates using membranes having pores sizes of 0.2 μm, 0.45 μm, 0.65 μm, 1.2 μm, 5 μm, 10 μm, and remarkably 20 μm (see, also, paragraph 12, of the Oelmüller declaration). Applicants' specification makes clear that the process of this invention is effective at providing high yields of not only large molecular DNA molecules (e.g., mammalian genomic DNA as in Examples 6 and 7, of the specification), but even much smaller RNA molecules from a variety of samples (e.g., as in Examples 1-5, of the specification). As noted above and as Dr. Oelmüller clearly explains in his declaration, any of the membranes in Table 5 of the specification that have pores larger than 0.100 μm would permit most nucleic acid molecules and other biomolecules to readily wash through if this method were simply a prior art ultrafiltration process as employed by Ogawa (see, paragraphs 13-15, of the Oelmüller declaration).

Applicants further note that a person skilled in this art who reads Ogawa is clearly taught that the ultrafiltration-based method of isolating phage DNA is a useful and successful alternative to other previously described DNA isolation methods that employ centrifugation, polyethylene glycol precipitation, phenol and chloroform extractions of proteins, and/or concentration by ethanol precipitation (see, e.g., column 1, lines 10-45, of Ogawa). In particular, in a comparison of a sample of phage DNA isolated according to the ultrafiltration-based protocol of Ogawa, a sample of a commercial preparation of phage DNA, and a sample of phage DNA purified by a polyethylene glycol precipitation protocol, Ogawa concludes that the ultrafiltration-based protocol successfully produced highly pure phage DNA (see, column 4, line 40-column 5, line 3, of Ogawa). Thus, there is no reason or motivation for the person of skill in the art who reads Ogawa to change the ultrafiltration-based protocol and devise Applicants' process of isolating and purifying nucleic acids that permits use of membranes with pore sizes that are much larger than those employed in ultrafiltration of biomolecules. In contrast, only Applicants' specification provides the teaching necessary for one to look back in hindsight and attempt to modify a prior art method, such as the ultrafiltration-based method of Ogawa, to

produce the process for isolating or purifying nucleic acids according to Applicants' claimed methods.

The Pfister reference is cited by the Examiner as an example of the RNeasy® products and protocols for isolationg RNA. Applicants have previously explained on this record that the RNeasy® products and protocols employ a silica-gel-based membrane for isolating RNA that does not permit isolation of the RNA from the same side of the membrane to which the RNA is applied. As Dr. Oelmüller explains in greater detail, the RNeasy® spin columns contain a silica membrane layer that is about 1.5 mm thick and is a fluffy, fibrous, fleece-like structured material. When liquid volumes of samples and elution buffer are applied to this silica material, such liquid is readily absorbed into this silica fleece making it *impossible* to harvest an eluate by a simple means such as pipetting from the top surface of the silica fleece membrane to which the sample was applied. Accordingly, the RNeasy® Mini-Handbook teaches retrieval of eluted RNA by centrifugation that forces the eluted RNA *through the silica fleece for collection on the other side of the silica fleece.* Moreover, Applicants' own specification also shows that silica fleece-like structured membranes do not provide high yields of RNA from the top side of the membrane to which the sample was applied and, thus, are not useful in the claimed invention (see, Example 1, page 17, lines 12-18, of the specification). Thus, as noted by Dr. Oelmüller, persons skilled in this art would not only readily distinguish the features and uses of the silica-gel-based RNeasy® products and protocols from the claimed invention but also would not have any reason to use the RNeasy® products and protocols to modify the ultrafiltration method of Ogawa to form the claimed process of Applicants' invention that employs highly porous, non-siliceous membranes (see, paragraph 16, of the Oelmüller declaration).

The Boom patent also fails to cure the deficiencies of the ultrafiltration-based protocol of Ogawa, whether considered alone or in combination with Pfister (RNeasy® products and protocols). As Dr. Oelmüller explains, the Boom patent simply demonstrates the previously accepted view in the prior art that nucleic acids bound to non-siliceous membranes could not be eluted in high yields and high purity from the same side of the membrane to which they were applied as in Applicants' claimed process (see, paragraph 17, of the Oelmüller declaration). Thus, the Boom patent can only suggest that the entire membrane containing the bound DNA be used as the source of the DNA. In this respect, the Boom patent can be seen as actually teaching

away from Applicants' claimed process in which purified nucleic acids are easily eluted and retrieved (e.g., by pipetting) in high yields from the same side of a highly porous, non-siliceous membrane to which a sample containing the nucleic acids was applied.

The above comments and the accompanying declaration of Dr. Oelmüller clearly show that Ogawa, either alone or in combination with Pfister (RNeasy®) and the Boom patent, fails to teach or suggest Applicants' claimed process that permits the isolation in high yields of nucleic acid molecules in a sample from the same side of a highly porous, non-siliceous membrane to which the sample was applied. Accordingly, the references, alone or in combination, do not support a *prima facie* case of obviousness under 35 USC § 103(a) for rejecting the claims. Applicants respectfully assert that without a reason for altering the working method according to Ogawa, the person of skill in the art would not make any alteration to the method and certainly not attempt to employ highly porous, non-siliceous membranes that clearly cannot function in ultrafiltration for size exclusion of biomolecules proposed as obvious by the Examiner. Accordingly, the rejections are in error, and the Examiner is respectfully requested to reconsider and withdraw the rejections.

Respectfully submitted,

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May 18, 2007
date

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